

Wounding increases glycolytic but not soluble sucrolytic activities in stored sugarbeet root

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Abstract

The wounding of sugarbeet (*Beta vulgaris* L.) roots by harvesting and piling operations increases the demand for sucrolytic and glycolytic products for wound-healing processes. To determine if sucrolytic and glycolytic enzyme activities increase to meet this demand and to identify those activities that may be induced, the activities of the major sucrolytic enzymes and the major regulatory enzymes of the glycolytic pathway were determined in wounded and unwounded sugarbeet roots during 13 days of storage at 10 °C. Activities of the enzymes responsible for catalysis of the first two reactions of the glycolytic pathway, hexokinase, fructokinase and phosphofructokinase, were elevated in wounded roots. Activities of the sucrolytic enzymes, sucrose synthase, alkaline invertase, and soluble acid invertase, and the glycolytic enzyme, pyruvate kinase, did not increase in wounded roots. The activities of the early glycolytic enzymes peaked 24–48 h after wounding when the demand for substrates for wound-healing processes was expected to be maximal. Fructokinase exhibited the greatest and most persistent increase in activity, increasing by 150%, 24 h after wounding and remaining elevated for the duration of the study. The increase in hexokinase, fructokinase, and phosphofructokinase activities suggests that expression of these early glycolytic enzymes may be up-regulated to meet the demand for glycolytic intermediates and products for wound-healing processes. The lack of an increase in any sucrolytic activities in response to wounding suggests that sucrolytic flux is not determined by the quantity of active protein present in the root, but by some undetermined mechanism.

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1. Introduction

Sugarbeet roots placed into storage are inevitably wounded from defoliation and the physical impacts incurred during harvest, transport, and piling. Typical injuries include bruising, root breakage, cracking, surface abrasions, cuts, and loss of small fragments (Wiltshire and Cobb, 2000). The degree of wounding varies by defoliation method, harvester type, harvester speed, and drop height in the loading and unloading of trucks, but is generally quite extensive (Parks and Peterson, 1979; Bentini et al., 2002). In a study using conventional harvesting and piling methods, 89% of the roots

placed into storage were bruised, 58% experienced breakage and loss of the lower tail portion of the root, and 38% were cracked (Steensen, 1996).

Wounding induces respiration, cell division, and biosynthesis of suberin, lignin, and plant defense compounds to aid in sealing wound sites off from the environment, repairing damaged tissues, and defending against opportunistic pathogens (reviewed in Lipetz, 1970; de Bruxelles and Roberts, 2001). In sugarbeet, the increase in metabolic activity occurs at the expense of sucrose (Cole, 1977; Akesson and Stout, 1978; Wyse, 1978) and two to three-fold greater loss of sucrose as a consequence of wounding has been documented in roots stored for 100–120 days (Wyse, 1978).

Sucrose utilization requires the activity of sucrolytic and glycolytic pathway enzymes to produce hexose phosphates

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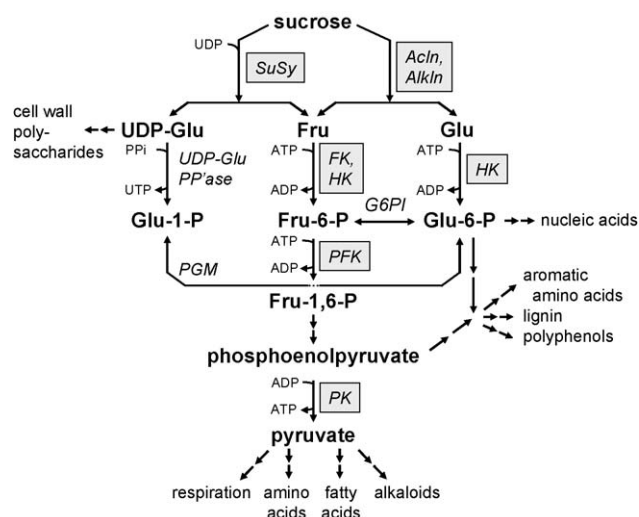


Fig. 1. Simplified schematic representation of sucrolytic and glycolytic pathways highlighting potential regulatory enzymes and illustrating the metabolic and biosynthetic fate of products. Enzymes implicated as having a role in the regulation of metabolic flux are highlighted with a shaded box. Single arrows (\rightarrow) indicate a single catalytic enzyme reaction. Double arrows (\rightleftharpoons) indicate multienzyme, multireaction pathways. Enzyme abbreviations: AcIn, acid invertase; AlkIn, alkaline invertase; FK, fructokinase; G6PI, glucose 6-phosphate isomerase; HK, hexokinase; PFK, phosphofructokinase; PGM, phosphoglucomutase; PK, pyruvate kinase; SuSy, sucrose synthase; UDP-Glu PPase, uridine 5'-diphosphate glucose pyrophosphorylase. Chemical compound abbreviations: Fru, fructose; Fru-6-P, fructose 6-phosphate; Fru-1,6-P, fructose 1,6-bisphosphate; Glu, glucose; Glu-1-P, glucose 1-phosphate; Glu-6-P, glucose 6-phosphate; PP_i, pyrophosphate; UDP, uridine 5'-diphosphate; UDP-Glu, uridine 5'-diphosphate glucose; UTP, uridine 5'-triphosphate.

and organic acids that are subsequently used for respiratory and biosynthetic reactions (Fig. 1). Sucrolytic enzymes cleave sucrose to hexose sugars, creating substrates for glycolysis and cell wall biosynthesis. Glycolytic enzymes oxidatively degrade hexoses to the three carbon organic acid, pyruvate, releasing energy and generating substrates for respiration and the biosynthesis of biological compounds including amino acids, fatty acids, nucleic acids, phenolic compounds, and alkaloids. Three sucrolytic enzymes, sucrose synthase (SuSy; EC 2.4.1.13), alkaline invertase (AlkIn; EC 3.2.1.26), and acid invertase (AcIn; EC 3.2.1.26), are found in mature sugarbeet root and contribute to the formation of hexose sugars (Berghall et al., 1997; Klotz and Finger, 2002). Fourteen glycolytic enzymes catalyze the 10 chemical reactions of glycolysis in plants, with the regulation of glycolytic flux believed to be primarily controlled by four enzymes (reviewed in Plaxton, 1996). These four enzymes, hexokinase (HK; EC 2.7.1.1), fructokinase (FK; EC 2.7.1.4), phosphofructokinase (PFK; EC 2.7.1.11), and pyruvate kinase (PK; EC 2.7.1.40) catalyze the initial two reactions and the final reaction of glycolysis.

Although wounded tissue has a greater demand for intermediates and products from sucrolytic and glycolytic pathways, it is not known how wounded sugarbeet tissue responds to meet this requirement. Metabolic flux can be controlled by

the quantity of enzyme present in a cell, substrate availability, the presence of enzyme inhibitors or activators, pH, and/or alteration of protein activity by changes in subunit association, phosphorylation, or oxidation status of disulfide bonds (Plaxton, 1996). To determine whether the enhanced demand for sucrolytic and glycolytic products in wounded roots is met by changes in enzyme activities and, additionally, to identify those enzyme activities that may increase to meet this demand, the activities of the major sucrolytic enzymes and the four glycolytic enzymes that have been implicated in the control of glycolytic flux in other plant species were determined in wounded and unwounded roots during 13 days of storage.

2. Materials and methods

2.1. Plant material and postharvest treatments

Sugarbeet hybrid VDH66156 (Van der Have, Rilland, Netherlands) was greenhouse grown in Sunshine Mix #1 (Sun Gro Horticultural Products, Seba Beach, Alberta, Canada) in 15 L pots with supplemental light under a 16-h light/8-h dark regime. Taproots were hand harvested 16–18 weeks after planting and gently hand washed. Roots were wounded by tumbling for 30 min in a pilot scale beet washer (Great Western batch washer; Hallbeck, 1982) immediately after harvesting and washing. Wounded and unwounded roots were incubated for 13 days at 10 °C and 90–95% relative humidity. Tissue located 1–2 cm beneath the epidermis at the point of maximum root girth was used for assays, with care taken to ensure that tissue excised from wounded roots was taken at the site of a visible bruise. The tissue was flash frozen in liquid nitrogen at time of sampling, lyophilized, ground to a fine powder, and stored at –80 °C prior to analysis. Four replicate roots were sampled at each time point from each treatment. Samples were taken immediately after harvest, at 24 h intervals for the first 7 days in storage, and after 9, 11 and 13 days in storage.

2.2. Protein extraction

Lyophilized tissue was homogenized in 12 volumes (w/v) of a solution containing 10 mM Na₂SO₃, 5 mM DTT, 1 mM MgCl₂, and 100 mM Hepes-NaOH, pH 7.2. The homogenate was passed over four layers of cheesecloth and centrifuged at 17,000 × g for 30 min. An aliquot of the supernatant was used for phosphofructokinase and pyruvate kinase activity assays. An additional aliquot of the supernatant was dialyzed against a solution of 1 mM DTT, 1 mM MgCl₂, and 10 mM Hepes-NaOH, pH 7.2 and used for fructokinase, hexokinase, and sucrose synthase activity assays. An equal volume of cold acetone was added to the remaining supernatant and precipitated proteins were pelleted by centrifugation at 10,000 × g for 15 min, washed with 50% cold acetone, resuspended in a solution containing 1 mM DTT, 1 mM MgCl₂, and 10 mM

Hepes-NaOH, pH 7.2, and used for acid and alkaline invertase activity assays. Acetone precipitation did not affect invertase specific activity, since similar specific activities for acid and alkaline invertases were observed for extracts concentrated by acetone precipitation or ultrafiltration (Klotz et al., 2003). All operations were performed at 4 °C.

2.3. Enzyme activity assays

Fructokinase, hexokinase, phosphofructokinase, and pyruvate kinase activities were determined by measuring the rate of change in absorbance at 340 nm and 25 °C using enzyme-coupled assays as described by Moorhead and Plaxton (1988). Assays for FK activity contained 1 mM ATP, 0.5 mM NAD⁺, 7 mM fructose, 5 mM MgCl₂, 2000 units L⁻¹ glucose-6-phosphate dehydrogenase, 6000 units L⁻¹ phosphoglucose isomerase, and 50 mM Hepes-NaOH, pH 7.5. Assays for HK activity contained 1 mM ATP, 0.5 mM NAD⁺, 7 mM glucose, 5 mM MgCl₂, 2000 units L⁻¹ glucose-6-phosphate dehydrogenase and 50 mM Hepes-NaOH, pH 7.5. Assays for PFK activity contained 0.2 mM ATP, 0.1 mM NADH, 1 mM fructose 6-phosphate, 5 mM MgCl₂, 1 mM EDTA, 1000 units L⁻¹ aldolase, 1000 units L⁻¹ triose phosphate isomerase, 5000 units L⁻¹ glycerol-3-phosphate dehydrogenase, and 50 mM Tris-HCl, pH 8.0. Assays for PK activity contained 2 mM ADP, 0.15 mM NADH, 2 mM PEP, 50 mM KCl, 10 mM MgCl₂, 2 mM DTT, 13000 units L⁻¹ lactate dehydrogenase, 0.2 g L⁻¹ bovine serum albumin and 50 mM Hepes-NaOH, pH 7.0. FK, HK, and PFK assays were corrected for NAD reductase or NADH oxidase activity by subtracting the rate of absorbance change when ATP was omitted from the reaction mixture. PK activity was corrected for contaminating NADH oxidase and phosphoenolpyruvate phosphatase activity by subtracting the rate of NADH oxidation when ADP was omitted from the reaction.

Sucrose synthase, acid invertase, and alkaline invertase activities were determined using spectrophotometric end point assays as previously described (Klotz and Finger, 2002). The reaction mixture for sucrose synthase contained 250 mM sucrose and 2 mM UDP in 100 mM MES, pH 6.5. The reaction mixture for invertase assays contained 100 mM sucrose and 100 mM NaOAc, pH 4.7 or 100 mM Hepes-NaOH, pH 8.0 for acid invertase and alkaline invertase activity assays, respectively. Control reactions were run on all samples by assaying in the absence of UDP for sucrose synthase assays or in the absence of sucrose for invertase assays.

Total protein was determined by the method of Bradford (1976) using bovine serum albumin as a standard. All enzyme and protein assays for each replicate ($n = 4$) were performed in duplicate.

2.4. Carbohydrate analysis

Sugar concentrations were determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) using lactose as an

internal standard. Sugar extraction and sample preparation have been previously described (Klotz and Finger, 2002). Samples were injected on to a 250 mm × 4 mm Dionex CarboPak PA-10 column (Sunnyvale, CA, USA) equipped with a 50 mm × 4 mm CarboPak PA-10 guard column, and eluted isocratically with 60 mM NaOH and 10 mM NaOAc at 16.7 $\mu\text{L s}^{-1}$. Carbohydrates were detected with a Dionex ED50 electrochemical detector operating in pulsed amperometric mode using the manufacturer's recommended settings for carbohydrate analysis and quantified using external standards.

3. Results

The impact of wounding on sugarbeet root sucrolytic and glycolytic activities was determined by comparing sucrose synthase, alkaline invertase, acid invertase, hexokinase, fructokinase, phosphofructokinase and pyruvate kinase activities in wounded and unwounded roots during 13 days of storage at 10 °C. Roots were wounded by tumbling in a pilot scale beet washer to mimic the type of injury caused by conventional harvesting and piling operations. Roots were severely injured to maximize wound responses (Fig. 2). Wounded roots incurred breakage and loss of the lower tail portion of the root, surface abrasions, and severe bruising. Wounded and unwounded roots were stored at 10 °C to mimic the temperature conditions typical at harvest in the Red River Valley of Minnesota and North Dakota, the major sugarbeet producing region in the USA.



Fig. 2. Representative roots from control (a) and wound (b) treatments after storage for 24 h at 10 °C. Roots were wounded by tumbling in a pilot scale beet washer resulting in breakage and loss of tail portion of the root, surface abrasions and severe bruising.

3.1. Sucrolytic enzyme activities

Sucrolytic activity in mature sugarbeet root is primarily due to sucrose synthase, alkaline invertase, and soluble acid invertase activities (Berghall et al., 1997; Klotz and Finger, 2002). Sucrose synthase catalyzes the reversible reaction of sucrose with uridine 5'-diphosphate (UDP) to form fructose and UDP-glucose, a metabolically active form of glucose that is a substrate for cell wall biosynthesis or glycolysis after its conversion to glucose 6-phosphate (Fig. 1). Alkaline invertase and acid invertase catalyze the irreversible hydrolysis of sucrose to glucose and fructose. Alkaline invertase is a soluble cytoplasmic enzyme with maximum activity at pH values of 7.0–8.0; acid invertase occurs as a soluble enzyme in the vacuole or as an insoluble enzyme in the cell wall with maximum activity in the pH range of 4.5–5.5. Activity of the insoluble isoform of acid invertase is barely detectable in mature and stored roots (Klotz and Finger, 2002, 2004) and was not determined in these studies.

Wounding caused no induction of any soluble sucrolytic activity (Fig. 3). Sucrose synthase (Fig. 3a), alkaline invertase (Fig. 3b), and soluble acid invertase (Fig. 3c) activities were not significantly higher in wounded roots than in unwounded controls at any time during 13 days of postwounding storage. Transient declines in sucrolytic activities in wounded roots relative to unwounded controls, however, were observed. Wounding was associated with transient declines in sucrose synthase activity 2, 7 and 9 days after harvest, alkaline invertase activity 3 days after harvest, and soluble acid invertase activity 2 days after harvest. Hexose concentrations were generally unchanged by wounding (Fig. 4). Glucose concentrations were not significantly different between wounded and unwounded control roots except for a transient increase in wounded roots 1 day after harvest (Fig. 4a). Fructose concentrations were not statistically different between wounded and unwounded control roots except for a transient decrease in wounded roots 7 days after harvest (Fig. 4b). No significant decline in sucrose concentration was evident in either control or wounded roots throughout the 13 days of the experiment (data not shown).

3.2. Glycolytic activities

Hexokinase and fructokinase catalyze the initial reaction of glycolysis—the irreversible, nucleotide triphosphate-dependent phosphorylation of the hexose sugars, glucose and fructose, to glucose 6-phosphate and fructose 6-phosphate (Fig. 1). HK and FK activities exhibit substrate specificities that allow independent regulation of glucose and fructose utilization (Renz and Stitt, 1993). Hexokinases are generally highly reactive with glucose and mannose and limitedly reactive with fructose; fructokinases react specifically with fructose (Doehlert, 1989; Renz and Stitt, 1993). Phosphofructokinase catalyzes the second reaction in glycolysis, the irreversible, ATP-dependent phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Pyruvate kinase cat-

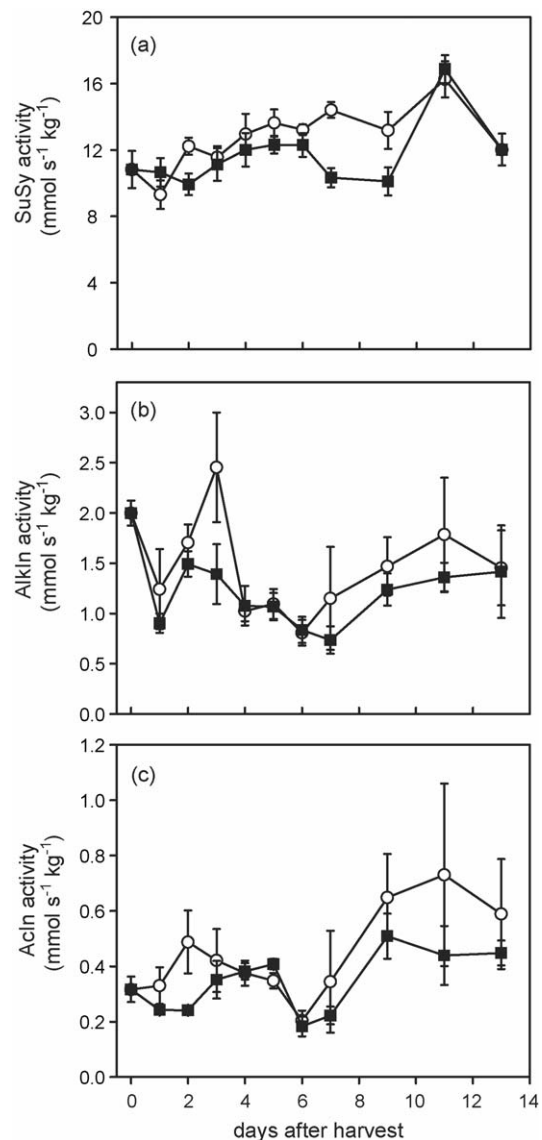


Fig. 3. Effect of wounding on sucrolytic enzyme activities during storage. Sucrose synthase (a), alkaline invertase (b), and acid invertase (c) activities of severely wounded (—■—) and unwounded control (—○—) roots were determined during 13 days of postharvest storage at 10 °C. Enzyme activity is expressed as $\text{mmol sucrose cleaved s}^{-1} \text{kg}^{-1}$ protein. Error bars are ± 1 standard error of the mean ($n = 4$), where these exceed the size of the symbol.

alyzes the final reaction of glycolysis, the irreversible transfer of a phosphate from phosphoenolpyruvate to ADP to form pyruvate and ATP.

Hexokinase activity was transiently elevated in wounded roots 1–2 days after wounding (Fig. 5a). Thirteen days after wounding, HK activity was greater in wounded roots relative to controls due to a decline in activity in the unwounded controls. Maximal HK activity occurred on the second day after wounding when HK activity increased nearly 60% over its activity at harvest. Fructokinase activity was persistently elevated in wounded roots throughout the 13 days storage period, with maximum FK activity 24 h after wounding (Fig. 5b). FK activity increased 150% from its activity at harvest after

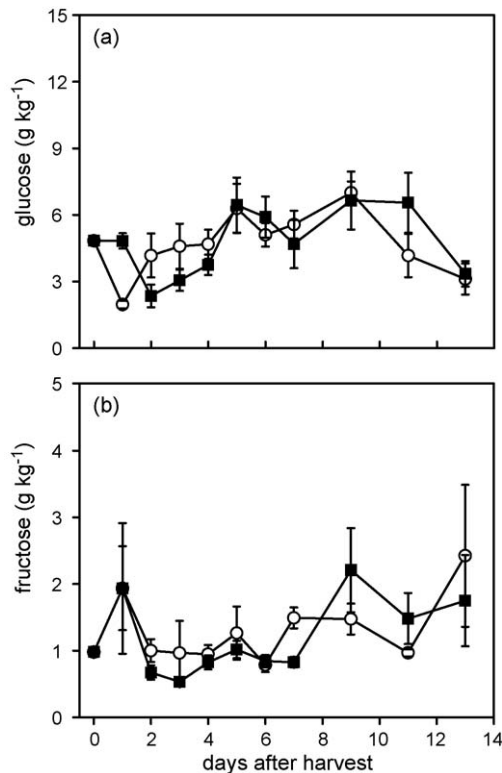


Fig. 4. Effect of wounding on glucose (a) and fructose (b) concentrations during storage. Carbohydrate concentrations of wounded (—■—) and unwounded control (—○—) roots were determined during 13 days of postharvest storage at 10 °C. Concentration is expressed as g kg^{-1} dry weight. Error bars are ± 1 standard error of the mean ($n=4$), where these exceed the size of the symbol.

1 day in storage, and was more than 60% higher than its activity at harvest throughout the 13 days in storage. Phosphofructokinase activity was transiently elevated in wounded roots 1–2 days after wounding and 9–11 days after wounding (Fig. 5c). The greatest elevation in PFK activity occurred 1–2 days after wounding when PFK activity increased by approximately 70% from its activity at harvest. Nine to eleven days after wounding, PFK activity in wounded roots was elevated by approximately 30% from its activity at harvest. Pyruvate kinase activity was more than 25-fold greater than HK, FK, and PFK activities and was unaffected by wounding (Fig. 5d). PK activity was not significantly greater in wounded roots than in unwounded controls at any time during 13 days of storage.

4. Discussion

Wounding was associated with an increase in the activities of the early glycolytic enzymes, hexokinase, fructokinase, and phosphofructokinase, but not with the late glycolytic enzyme, pyruvate kinase, or any soluble sucrolytic activity. The increases in HK, FK and PFK activities were greatest 1–2 days after injury when wound-induced respiration (Dilley et al., 1970; Wyse and Peterson, 1979) and defense protein gene expression (Schweizer et al., 1998; Graham et al., 2003) are maximal and the demand for glycolytic intermediates was expected to be greatest. Elevations in FK and PFK activities were also observed 9 days after wounding, coincidental with the onset of cell wall lignification (Ibrahim et al., 2001). HK, FK, and PFK have been implicated in the control of

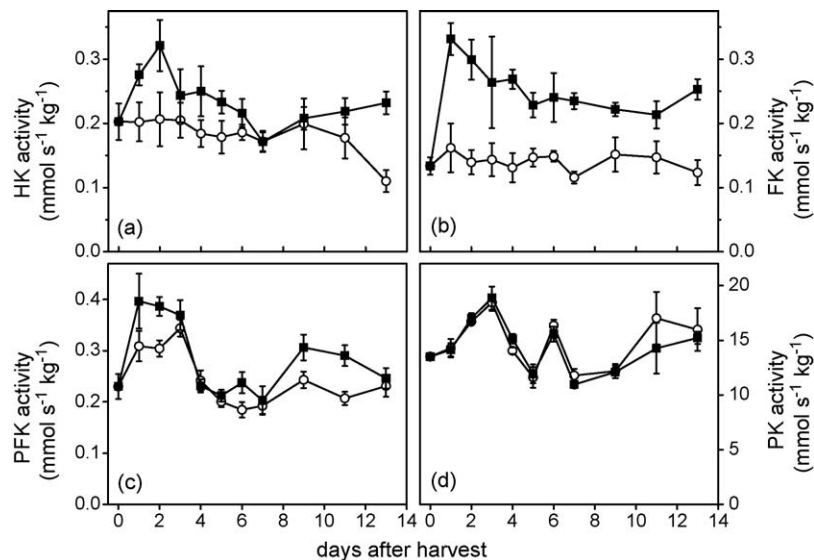


Fig. 5. Effect of wounding on the activities of the major regulatory enzymes of the glycolytic pathway during storage. Hexokinase (a), fructokinase (b), phosphofructokinase (c), and pyruvate kinase (d) activities of severely wounded (—■—) and unwounded control (—○—) roots were determined during 13 days of postharvest storage at 10 °C. Enzyme activities are expressed as $\text{mmol of product formed s}^{-1} \text{kg}^{-1}$ protein. Error bars are ± 1 standard error of the mean ($n=4$), where these exceed the size of the symbol.

glycolytic flux in other plant systems (Kubota and Ashihara, 1990; reviewed in Plaxton, 1996). The increase in HK, FK and PFK activities and the timing of their induction relative to wound-healing processes suggests that these early glycolytic enzymes may be up-regulated to provide for the increased demand for glycolytic substrates in wounded sugarbeet roots. Pyruvate kinase, the terminal enzyme in the glycolytic pathway, has also been implicated in the control of glycolysis in other plant species (reviewed in Plaxton, 1996). PK activity was not elevated in wounded sugarbeet roots and was more than 25-fold greater than HK, FK and PFK activities in both wounded and unwounded roots. The greater activity of PK relative to the early glycolytic enzymes and its unchanging activity in response to wounding suggest that this enzyme is not limiting in sugarbeet root.

The greatest and most persistent increase in activity in response to wounding was exhibited by fructokinase. FK activity increased by 150%, 1 day after wounding and was elevated throughout the entire 13 day storage period. Increased FK activity has also been reported in postharvest sugarbeet roots stressed by high temperatures and dehydrating conditions (Sakalo and Tyltu, 1997). FK activity has been implicated in contributing to the control of glycolytic flux in several plant systems including developing potato tubers (Viola, 1996), anoxic maize root tips (Bouny and Saglio, 1996), and periwinkle suspension-cultured cells (Kubota and Ashihara, 1990). While the function of fructokinase in glycolytic control in sugarbeet roots remains to be determined, the extent of its activation in wounded roots suggests that FK may have a key role in supporting the increased metabolic activity of wounded sugarbeet root.

The activities of the soluble sucrolytic enzymes, sucrose synthase, alkaline invertase, and acid invertase, were generally unchanged by root injury, although short transient declines of 1 or 2 days duration were observed for each activity. Previously, Vaccari et al. (1988) reported transient increases of 30 and 40% for soluble acid invertase and alkaline invertase activities, respectively, in wounded roots, while Rosenkranz et al. (2001) reported a greater than 700% increase in soluble acid invertase activity in wounded tissue. The cause of the disparate results between this and previous studies is unknown, but is thought to relate to differences in storage conditions, type of injury, and tissue source in the different studies. In the present study, analyses were conducted on wound tissue collected from roots that were mechanically bruised and stored at 10 °C and 90–95% relative humidity. In contrast, Vaccari et al. (1988) determined enzymatic changes in whole roots that had been mechanically injured and stored on the ground under ambient conditions that included 6 cm of rain and temperatures that fluctuated from 14 to 27 °C. In the study conducted by Rosenkranz et al. (2001), tissue slices, rather than whole roots, were the experimental unit of study and these were stored at room temperature and high humidity. The transient declines in sucrolytic activities observed in the present study were not observed in the studies of

Vaccari et al. (1988) and Rosenkranz et al. (2001). A decline in sucrose synthase mRNA in the initial 24 h after wounding, however, has been reported in sugarbeet root and potato tuber (Salanoubat and Belliard, 1989; Hesse and Willmitzer, 1996). The cause for the transient declines in sucrolytic activities is unknown, but they may be by-products of the massive change in metabolism that occurs in wounded tissue as plant resources are redirected to the synthesis of proteins needed for wound-healing and defense responses (de Bruxelles and Roberts, 2001).

Sucrose and hexose concentrations were generally unaffected by wounding. Although sucrose is the principal substrate for respiration in sugarbeet root (Barbour and Wang, 1961), no decline in sucrose concentration was observed in either wounded or control roots throughout the storage period. Other storage studies with sugarbeet roots have similarly failed to detect a loss of sucrose during storage (Rosenkranz et al., 2001; Klotz and Finger, 2004). Sucrose loss was not detected presumably because the quantity of sucrose catabolized was small relative to the concentration of sucrose in roots and was less than the natural variability in sucrose concentration among replicates. Hexose concentrations were also generally unchanged during storage of wounded and unwounded roots. In previous studies, hexose concentrations have been observed to decrease nearly 3-fold during storage of wounded roots (Vaccari et al., 1988) or increase more than 10-fold in wounded tissue (Rosenkranz et al., 2001). The relatively constant concentrations of hexose sugars in this study suggest that the rate of hexose production via sucrolytic reactions matched the rate of hexose degradation via glycolytic reactions in both wounded and unwounded sugarbeet roots. In wounded roots, the elevated demand for hexose sugars, therefore, was likely met with an elevated production of hexose sugars by sucrolytic activities. In plant cells, metabolic flux through a pathway can be controlled by the quantity of active enzyme present, substrate availability, product concentration, pH conditions, and/or the concentration of compounds that stimulate or inhibit activity (Plaxton, 1996). Since sucrolytic activities did not increase in wounded roots, any increase in the production of hexose sugars was achieved by a mechanism other than an alteration in active sucrolytic enzyme levels.

Sucrose catabolism in sugarbeet root is thought to be predominantly catalyzed by sucrose synthase (Echeverría and Gonzalez, 2003; Klotz and Finger, 2004). SuSy is the predominant sucrolytic activity in mature and postharvest sugarbeet roots (Klotz and Finger, 2004) and catalyzes a reversible reaction with an *in vitro* equilibrium constant that favors sucrose synthesis over degradation (Cardini et al., 1954). Because of these physical and kinetic properties, sucrolytic flux through the SuSy-catalyzed reaction can be altered without a change in active protein levels via a shift in the enzyme's reaction equilibrium. Removal of reaction products by a downstream enzyme, such as fructokinase, would have the 2-fold effect of shifting SuSy's reaction equilibrium toward sucrose degradation and rendering the SuSy-catalyzed reac-

tion irreversible. Regulation of sucrose breakdown by the coupling of SuSy and FK activities has been proposed in potato tubers (Viola, 1996). If such a mechanism occurs in sugarbeet root, the increased FK activity observed in wounded roots may cause an increase in the rate of sucrose degradation by SuSy.

The impact of wounding on sucrolytic and glycolytic activities suggest that the early glycolytic enzymes, hexokinase, fructokinase and phosphofructokinase, may have a role in up-regulating the flux of carbon compounds through sucrolytic and glycolytic pathways to support wound-healing processes. The association between these activities and wound responses, however, is not proof of function, and additional research is needed to clarify the impact of these enzymes on the availability of substrates for biosynthetic and respiratory processes. Similarly, a role for fructokinase in promoting sucrose degradation by influencing the equilibrium of sucrose synthase reaction remains to be established but will be examined in future research.

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